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## THE EFFECT OF VARIOUS CENTRALLY ACTIVE DRUGS ON ADENOSINE UPTAKE BY THE CENTRAL NERVOUS SYSTEM.

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**Abstract**—1. Adenosine and its analogs depress the firing of neurons in various brain regions. The primary mode of action of adenosine in exerting this effect appears to be the depression of transmitter release from presynaptic nerve terminals. This is a result of reduced calcium mobilization.  
2. Adenosine uptake inhibitors and deaminase inhibitors depress the firing of central neurons. Adenosine antagonists, caffeine and theophylline, excite central neurons. Adenosine is therefore likely to be released in sufficient quantities to exert an ongoing modulation of synaptic transmission in the intact brain.  
3. A number of groups of centrally active drugs inhibit adenosine uptake by brain synaptosomal preparations. These include the benzodiazepines, phenothiazines, various other sedatives and hypnotics, tricyclic antidepressants, non-steroidal anti-inflammatory analgesics, some steroids, diphenylhydantoin, puromycin and toyocamycin.  
4. It is proposed that many agents with anxiolytic, sedative, analgesic or anti-convulsant actions may achieve their effects by inhibiting adenosine uptake and thus potentiating extracellular adenosine levels.  
5. Morphine also elevates extracellular adenosine levels but achieves this by enhancing adenosine release.

### INTRODUCTION

During the past few years it has become apparent that adenosine inhibits transmission at various central synapses and can potentially depress the firing of single neurons in the brain. These studies, which have involved iontophoretically applied adenosine and *in situ* neurons or adenosine applied topically onto brain slices, have provided an explanation for the previously observed behaviorally depressant effects of intracerebroventricularly administered adenosine (Feldberg & Sherwood, 1954; Buday *et al.*, 1961).

Iontophoretic studies have demonstrated that adenosine and its analogs can depress the spontaneous firing of neurons in several regions of the brain, including the cerebral (Fig. 1) and cerebellar cortices, hippocampus and caudate nucleus (Phillis *et al.*, 1979; Phillis & Wu, 1981). In a study on spinal cord Renshaw cells Lekić (1977) was able to demonstrate that whereas adenosine could depress cholinergic synaptic activation elicited by ventral root stimulation, it did not alter the effects of iontophoretically applied acetylcholine (ACh). This experiment provided a convincing demonstration of the presynaptic locus of action of adenosine. Studies on the peripheral and central (CNS) nervous systems have confirmed that adenosine is able to reduce ACh release from presynaptic terminals (Ginsborg & Hirst, 1972; Jhamandas & Sawynok, 1976). Adenosine's actions are not restricted to cholinergic nerve terminals as it has been shown to depress the K<sup>+</sup>-evoked release of labelled noradrenaline from cerebral cortical slices and of labelled dopamine and serotonin from striatal slices (Harms *et al.*, 1978, 1979). It also has a weakly de-

pressant action on the release of labelled  $\gamma$ -aminobutyric acid (Hollins & Stone, 1980).

The mechanism by which adenosine reduces transmitter release has been investigated in some detail and appears to involve a reduction in the availability of calcium. This may be a result either of a decreased influx of calcium across the depolarized terminal membrane (Kuroda, 1978; Ribeiro *et al.*, 1979) or of a sequestration of intracellular calcium (Branisteanu *et al.*, 1979). There is evidence from both peripheral and central synapses that an increase in extracellular calcium levels will reduce the effects of adenosine.

The depressant effect of iontophoretically applied adenosine on the firing of cerebral cortical neurons was enhanced by inhibitors of adenosine uptake (dipyridamole, hexobendine, papaverine) and antagonized by the methylxanthines, caffeine and theophylline. An interesting observation was that inhibitors of adenosine uptake powerfully depressed neuronal firing, even when no adenosine had been applied, and that the methylxanthines frequently accelerated neuronal discharges (Phillis *et al.*, 1979). A clear implication of these results is that central neurons are under the continuing depressant influence of endogenously released adenosine. Accompanying the realization that extracellular adenosine levels might be an important factor in the control of CNS excitability, there has been an increasing awareness of the possibility that many centrally active drugs might exert some of their actions by purine-linked mechanisms.

Our observations on an antagonism between adenosine and the methylxanthines led to the proposal (Phillis & Kostopoulos, 1975) that the central stimulant actions of caffeine and theophylline result from their blockade of the depressant effects of endogenously released adenosine. This explanation has since been widely accepted (Fredholm, 1980; Cardinali,

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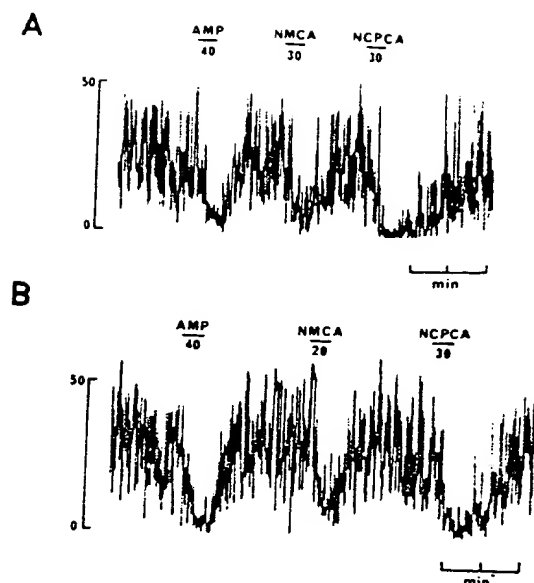


Fig. 1. Firing frequency records from two different cerebral cortical neurons in a nitrous oxide and methoxyflurane anaesthetized rat. This is a rate-meter record of the spontaneous firing, with the number of action potentials per second on the ordinate. Horizontal bars above the record indicate periods of drug application. AMP (40 nA), adenosine 5'-methylcarboxamide (NMCA, 30 nA) and adenosine 5'-cyclopropylcarboxamide (NCPA, 30 nA) depressed the firing of the neuron in trace (A) to an approximately equivalent extent. Similar results were obtained with the second neuron (trace B).

1980). The ingestion of large amounts of caffeine is associated with symptoms of anxiety (Greden, 1974) indicating that adenosine may act as an endogenous substrate for the control of anxiety and depression. Our interest has therefore been focussed on the possibility that sedative and anti-anxiety agents might exert their effects by enhancing purinergic control of neuronal excitability. Data obtained during the past two years do indeed suggest that a number of drugs with sedative, anxiolytic and analgesic properties may act, in part, by elevating the extracellular levels of adenosine. This effect may be a result either of an inhibition of adenosine uptake (phenothiazines, benzodiazepines, antidepressants and analgesics) or of an increased release of adenosine from the tissues (morphine).

The abilities of 99 centrally active agents to inhibit adenosine uptake by rat brain cerebral cortical synaptosomes are presented in Table 1. Many of these compounds have sedative properties and are also known to be potent inhibitors of the enzyme phosphodiesterase (Weinryb *et al.*, 1972; Daly, 1977).

In the light of this relationship between sedative/anxiolytic activity and ability to inhibit phosphodiesterase, it was proposed that drugs which reduce anxiety may do so by inhibiting phosphodiesterase and thus enhancing brain cyclic AMP levels (Goodsell *et al.*, 1971; Beer *et al.*, 1972; Hess *et al.*, 1975). This hypothesis was, however, developed before the central actions of adenosine had been recognized and we have subsequently proposed (Phillis & Wu, 1981) that the anxiolytic, sedative and analgesic properties of certain therapeutic agents may result from their

abilities to inhibit the uptake of endogenously released adenosine. The resulting enhanced levels of adenosine would depress arousal anxiety and interfere with the transmission of nociceptive information.

The various groups of substances listed in Table 1 will be discussed in this contribution.

#### ADENOSINE UPTAKE STUDIES

##### *Phenothiazines and related antipsychotics*

The phenothiazines were originally developed as sedative agents and were also used for pre-operative medication (Baldessarini, 1980). The frequent occurrence of extrapyramidal disorders during the continued administration of agents in this group has limited their widespread use as sedatives and anxiolytics and they are now used primarily as anti-psychotic agents. It has been hypothesized that they exert their effect by acting as dopamine receptor antagonists. Unlike their sedative actions which become evident shortly after administration and tend to become less pronounced with repeated administration, the anti-psychotic effects of the phenothiazines develop slowly over a period of days or weeks. The anti-psychotic actions of the phenothiazines are therefore likely to be mediated by a different mechanism to their sedative effects.

Trifluoperazine, spiroperidol, penfluridol and sulpiride were the most active adenosine uptake inhibitors in this group with  $IC_{50}$  values in the low micromolar or submicromolar range. Fluphenazine, thioridazine, 2-chlorphenothiazine (chlorpromazine),  $\alpha$ -flupenthixol, haloperidol and pimozide were also potent inhibitors of adenosine uptake with  $IC_{20}$  values in the submicromolar range. The significance of  $IC_{20}$  values has previously been demonstrated in peripheral tissues in which a 20% reduction in adenosine uptake was shown to double the action of exogenously applied adenosine (Hopkins, 1973).

Chlorpromazine was considerably more active than its 1-, 3- and 4- analogs, which lack tranquillizer activity (Green, 1967).  $\alpha$ -Flupenthixol, which possesses pharmacological activity, was more potent than  $\beta$ -flupenthixol, which has little activity in pharmacological screening tests (Møller-Nielsen *et al.*, 1973).

These results are interesting in that they indicate that at therapeutic plasma levels (Curry *et al.*, 1970) the phenothiazines should be present in sufficient concentrations to significantly affect adenosine uptake.

##### *Benzodiazepines*

The benzodiazepines are a widely used group of drugs with anticonvulsant, hypnotic, anxiolytic and muscular relaxant properties. An interaction between the benzodiazepines and adenosine was demonstrated in pharmacological experiments in which diazepam was observed to potentiate the depressant effects of iontophoretically applied adenosine on the firing of cerebral cortical neurons (Phillis, 1979). Further experiments revealed that the effects of flurazepam on neuronal firing were antagonized by theophylline (Phillis *et al.*, 1979). Since benzodiazepines do not interact with the adenosine receptor (Williams *et al.*, 1981) this observation suggested that flurazepam was exerting its effects by potentiating the extracellular levels of endogenously released adenosine. Caffeine

has been shown to antagonize, in a selective manner, several of the central effects of diazepam (Polc *et al.*, 1981).

A study of the effects of diazepam on  $^3\text{H}$ -adenosine and acetylcholine release from the rat cerebral cortex has demonstrated that benzodiazepines enhance the efflux of labelled purines and at the same time depress the release of acetylcholine (Phillis *et al.*, 1980). The depression of acetylcholine release was blocked by a prior administration of theophylline, indicating that it was secondary to the increase in extracellular adenosine levels.

The abilities of a series of benzodiazepines to inhibit adenosine uptake by rat brain cortical synaptosomes are presented in Table 1. The potencies of the benzodiazepines as adenosine uptake inhibitors show a good correlation with their clinical, anti-conflict (Fig. 2) and receptor binding potencies, implying that inhibition of adenosine uptake is an important factor in the central actions of the benzodiazepines.

Adenosine uptake is a carrier mediated process and if benzodiazepine binding sites overlap with adenosine uptake sites, it would be expected that adenosine uptake inhibitors should inhibit benzodiazepine binding to cell membranes. A survey of the actions of several competitive inhibitors of adenosine uptake has shown that these substances are indeed competitive inhibitors of  $^3\text{H}$ -diazepam binding to brain membrane receptors (Fig. 3). The converse also holds in that benzodiazepines can displace a potent adenosine uptake inhibitor 6-(2-hydroxy-5-nitrobenzyl)-thioinosine from its binding sites on erythrocytes (Clanachan *et al.*, 1982).

A role for adenosine in benzodiazepine action therefore appears a likely possibility and the purine-link mechanism may act in concert with other proposed mechanisms of benzodiazepine action (e.g. GABA- and serotonin-linked mechanisms).

#### Non-benzodiazepine anxiolytics and sedatives

This group of compounds includes a number of potent inhibitors of brain phosphodiesterase such as the pyrazolopyridines (SQ 20-009 and SQ 66-007), a 3,4-dialkoxybenzyl-2-imidazolidinone (RO 20-1724), a 3,4-dialkoxyphenyl-2-pyrrolidone (ZK 62711) and a triazolopyrimidine (ICI 63,197) which are central depressants (Daly, 1977). Others (meprobamate, pentobarbital), which also have sedative actions, are poor inhibitors of brain phosphodiesterases (Weinryb *et al.*, 1972; Beer *et al.*, 1972).

Many of the anxiolytics and sedatives in this group were potent inhibitors of adenosine uptake by rat brain synaptosomes. These included meprobamate, thalidomide, SQ 20-009, RO 20-1724, rolipram, zopiclone [6-(5-chloro-2-pyridyl)-6,7-dihydro-7-oxo-5H-pyrrolo[3,4-b]pyrazine-5-yl]-4-methyl-1-piperazine carboxylate (Blanchard *et al.*, 1979), CL 218,872 [3-methyl-6-[3-(trifluoromethyl)phenyl]-1,2,4-triazolo[4,3-b]pyridazine] (Lippa *et al.*, 1979) and ICI 63,197. Methaqualone, pentobarbital, buspirone and ethanol were poor inhibitors of adenosine uptake. The results with buspirone are particularly interesting in that this compound reportedly has a selective anti-anxiety action without any accompanying sedative, anticonvulsant, or muscle relaxant effects (Stanton *et al.*, 1981). Buspirone is thought to exert its anxiolytic

effects by acting as an agonist at central dopamine receptors.

#### Anticonvulsants

Three agents are listed in this category. Phenobarbital and carbamazepine were very weak inhibitors of adenosine uptake whereas diphenylhydantoin had an  $\text{IC}_{20}$  of  $1.5 \times 10^{-6}$  M. The three compounds are poor inhibitors of brain phosphodiesterase (Weinryb *et al.*, 1972; Beer *et al.*, 1972; Palmer, 1979). Our findings suggest that of the three substances tested in this category, only diphenylhydantoin action is likely to involve an elevation of extracellular adenosine levels.

#### Steroids

Forty years have passed since Selye (1942) described the anaesthetic actions of steroid hormones such as progesterone, testosterone and corticosterone. The findings presented in Table 1, which demonstrate that many of the steroid hormones are relatively potent inhibitors of adenosine uptake ( $\text{IC}_{20}$  values in the range  $10^{-6}$ – $10^{-5}$  M) suggest that potentiation of extracellular adenosine levels may be involved. Relatively high doses of progesterone (54 mg/kg) must be administered to induce anaesthesia (Selye, 1942) and it can be anticipated that systemic concentrations would initially exceed  $10^{-5}$  M. This could result in a significant elevation of brain extracellular adenosine levels. 17- $\beta$ -Estradiol, 17- $\beta$ -ethinylestradiol, diethylstilbestrol and dexamethasone were approximately equipotent with progesterone. Alphaxalone, a major component of the steroid anaesthetic, Alfathesin (Glaxo) was rather less potent as an adenosine uptake inhibitor. Tetrahydrocortisone was even less active.

The observation that steroids can inhibit adenosine uptake is of considerable interest in that it offers an explanation for the hypnotic and anaesthetic activities of steroids in the pregnane class (Selye, 1942) and may account for some of the anti-inflammatory actions of the glucocorticoids (Glenn *et al.*, 1963; Jasani, 1979). Oestrogens and progestogens can be quite potent inhibitors of rat brain phosphodiesterase (Weinryb *et al.*, 1972).

#### Antidepressant agents

Caffeinism has been related to clinical depression (Greden *et al.*, 1978) suggesting that adenosine may, in some fashion, be involved in this syndrome. It was therefore of considerable interest when the antidepressant desipramine was shown to increase cyclic AMP levels in guinea-pig cortical tissues by a theophylline-sensitive mechanism (Kodama *et al.*, 1971). Subsequent studies have shown that a number of antidepressant agents, at concentrations of 200–500  $\mu\text{M}$ , increase the cyclic AMP content of guinea-pig cortical tissues and that this effect is antagonized by theophylline (Huang & Daly, 1972; Sattin *et al.*, 1978). The most active of the compounds tested were chlorimipramine, desipramine, nortriptyline and iprindole. When applied iontophoretically, these substances potentiated the depressant actions of adenosine on the firing of rat cerebral cortical neurons and the suggestion was made that these compounds may act by reducing the uptake or metabolism of applied adenosine, or alternatively by releasing small amounts of adenosine.

The studies summarized in Table I show that tricyclic antidepressants can inhibit uptake of adenosine by rat brain synaptosomes. However, with the exception of nortriptyline, which had an  $IC_{20}$  of  $8.0 \times 10^{-7}$  M, the tricyclic antidepressants were rather weak inhibitors of adenosine uptake ( $IC_{20}$  values of  $10^{-5}$ – $10^{-4}$  M). A non-tricyclic antidepressant, viloxazine, had little effect on adenosine uptake.

The therapeutic plasma concentration of tricyclic antidepressants in patients is around 150  $\mu$ g/ml or 0.5  $\mu$ M (Kinard *et al.*, 1978) and animal experiments suggest that brain levels may be considerably higher (Biegon & Samuel, 1979). It is conceivable therefore that inhibition of adenosine uptake may be a factor in antidepressant therapy and in patients treated with antidepressants to alleviate chronic pain (Charpentier, 1966; Budd, 1978).

#### Antihistamines

Central depression (sedation, drowsiness) is a frequent side-effect of the administration of therapeutic

doses of the histamine  $H_1$  antagonists. Several such antagonists were tested during the course of our studies, but none of these had effects on adenosine uptake by rat brain synaptosomes.

#### Coronary vasodilators

A number of coronary vasodilators were tested as adenosine uptake inhibitors. Dilazep, hexobendine, lidoflazine, dipyridamole and three analogs of dipyridamole (RE 244-BS, RE 642-BS and RE 86-BS) proved to be potent inhibitors of adenosine uptake with  $IC_{20}$  values of  $10^{-9}$ – $4.0 \times 10^{-8}$  M. Papaverine and prenylamine were somewhat less potent.

Three of the so-called "calcium antagonists" coronary vasodilators (verapamil, methoxyverapamil (D600) and diltiazem) were only weak inhibitors of adenosine uptake with  $IC_{20}$  values  $> 10^{-5}$  M. This lack of effect of the calcium antagonists implies that the actions of agents such as dipyridamole and dilazep cannot readily be attributed to an effect on membrane calcium binding and/or permeability.

Table I. Effects of drugs on  $^3H$ -adenosine uptake by rat brain synaptosomes\*

Therapeutic category	Class of drug	Drug	$IC_{20}$ (M)	$IC_{50}$ (M)
Antipsychotic Anxiolytic Sedative	Phenothiazine	Trifluoperazine	$2.0 \times 10^{-9}$	$1.1 \times 10^{-6}$
		Fluphenazine	$6.0 \times 10^{-7}$	$1.0 \times 10^{-5}$
		Thioridazine	$5.0 \times 10^{-7}$	$1.0 \times 10^{-4}$
		1-Chlorphenothiazine	$1.0 \times 10^{-5}$	$> 10^{-4}$
		2-Chlorphenothiazine	$5.0 \times 10^{-7}$	$1.5 \times 10^{-4}$
		3-Chlorphenothiazine	$1.5 \times 10^{-5}$	$> 10^{-4}$
		4-Chlorphenothiazine	$1 \times 10^{-4}$	
	Thioxanthene	$\alpha$ -Flupenthixol	$3.7 \times 10^{-7}$	$1.1 \times 10^{-5}$
		$\beta$ -Flupenthixol	$1.0 \times 10^{-5}$	$6.0 \times 10^{-5}$
	Butyrophenone	Haloperidol	$4.0 \times 10^{-7}$	$> 10^{-4}$
		Spiroperidol	$1.6 \times 10^{-9}$	$7.0 \times 10^{-7}$
	Diphenylbutylpiperidine	Pimozide	$1.0 \times 10^{-7}$	$> 10^{-4}$
		Penfluridol	$1.1 \times 10^{-7}$	$5.6 \times 10^{-6}$
	Dibenzodiazepine	Clozapine	$2.8 \times 10^{-6}$	$5.0 \times 10^{-5}$
	Sulfamoylbenzamide	Sulpiride	$1.0 \times 10^{-9}$	$8.5 \times 10^{-7}$
Anxiolytic Hypnotic Sedative	Benzodiazepine	Clonazepam	$5.0 \times 10^{-9}$	$3.5 \times 10^{-5}$
		Nitrazepam	$3.0 \times 10^{-8}$	$5.0 \times 10^{-4}$
		Lorazepam	$6.0 \times 10^{-8}$	$4.0 \times 10^{-5}$
		Ro 11-6896	$8.4 \times 10^{-8}$	$6.0 \times 10^{-5}$
		Diazepam	$9.0 \times 10^{-8}$	$7.0 \times 10^{-5}$
		Flunitrazepam	$1.6 \times 10^{-7}$	$1.0 \times 10^{-3}$
		Medazepam	$2.6 \times 10^{-7}$	$2.3 \times 10^{-4}$
		Flurazepam	$2.7 \times 10^{-7}$	$1.3 \times 10^{-3}$
		Bromazepam	$1.1 \times 10^{-6}$	$3.5 \times 10^{-4}$
		Chlordiazepoxide	$3.7 \times 10^{-6}$	$1.5 \times 10^{-3}$
		Ro 5-3636	$6.0 \times 10^{-6}$	$6.0 \times 10^{-3}$
		Oxazepam	$9.0 \times 10^{-6}$	$2.5 \times 10^{-4}$
		Ro 11-6893	$2.0 \times 10^{-5}$	$> 10^{-4}$
	Non-Benzodiazepine Anxiolytics and Sedatives	Meprobamate	$1.2 \times 10^{-7}$	$5.5 \times 10^{-5}$
		Methaqualone	$> 10^{-4}$	—
		Thalidomide	$6.0 \times 10^{-7}$	$> 10^{-4}$
		Pentobarbital	$7.0 \times 10^{-5}$	$1 \times 10^{-3}$
		Bupirone HCl	$2.0 \times 10^{-5}$	$> 10^{-4}$
		SQ 20-009	$1.8 \times 10^{-8}$	$1.4 \times 10^{-6}$
		SQ 66-007	$1.1 \times 10^{-5}$	$6.0 \times 10^{-5}$
		RO 20-1724	$1.0 \times 10^{-8}$	$> 10^{-4}$
		Rolipram (ZK 62711)	$1.0 \times 10^{-7}$	$1.0 \times 10^{-3}$
		Zopiclone	$1.0 \times 10^{-7}$	$7.5 \times 10^{-5}$
		CL 218, 872	$1.6 \times 10^{-8}$	$4.6 \times 10^{-5}$
		ICI 63,197	$1.6 \times 10^{-7}$	$> 10^{-4}$
		Ethanol	$> 10^{-3}$	

Table 1. —continued

Therapeutic category	Class of drug	Drug	IC <sub>25</sub> (M)	IC <sub>50</sub> (M)
Anticonvulsants		Phenobarbital	$1.0 \times 10^{-3}$	$> 10^{-3}$
		Carbamazepine	$1.7 \times 10^{-4}$	$> 10^{-3}$
		Diphenylhydantoin	$1.5 \times 10^{-6}$	$2.0 \times 10^{-4}$
Steroids		Dexamethasone acetate	$6.0 \times 10^{-6}$	$> 10^{-4}$
		Progesterone	$5.8 \times 10^{-6}$	$4.6 \times 10^{-5}$
		17 $\beta$ -Estradiol	$5.0 \times 10^{-6}$	$4.5 \times 10^{-5}$
		17 $\alpha$ -Ethinylestradiol	$6.6 \times 10^{-6}$	$7.4 \times 10^{-5}$
		Diethylstilbestrol dipropionate	$4.8 \times 10^{-6}$	$5.0 \times 10^{-5}$
		Alphaxalone	$4.0 \times 10^{-5}$	$> 10^{-3}$
		Tetrahydrocortisone	$1.0 \times 10^{-4}$	
Antidepressant	Tricyclic	Imipramine	$> 10^{-4}$	
		Chlorimipramine	$2.6 \times 10^{-5}$	$1.7 \times 10^{-4}$
		Ipindole	$1.0 \times 10^{-5}$	$> 10^{-4}$
		Amoxapine	$1.7 \times 10^{-5}$	$> 10^{-4}$
		Nortriptyline HCl	$8.0 \times 10^{-7}$	$> 10^{-4}$
	Non-Tricyclic	Viloxazine	$> 10^{-4}$	
		Zimelidine	$5.0 \times 10^{-5}$	$> 10^{-4}$
Antihistamines		Cinnarizine	$1.0 \times 10^{-4}$	
		Diphenhydramine	$> 10^{-4}$	
		Chlorcyclizine	$> 10^{-4}$	
		Cyclizine	$> 10^{-4}$	
		Tripeleminamine	$> 10^{-4}$	
Coronary Vasodilators		Hexobendine	$2.3 \times 10^{-8}$	$1.2 \times 10^{-5}$
		Lidoflazine	$4.0 \times 10^{-8}$	$1.4 \times 10^{-4}$
		Papaverine	$2.9 \times 10^{-7}$	$2.9 \times 10^{-5}$
		Dilazep	$1.0 \times 10^{-9}$	$1.5 \times 10^{-6}$
		Prenylamine	$5.0 \times 10^{-6}$	$1.5 \times 10^{-4}$
		Dipyridamole	$2.5 \times 10^{-9}$	$4.5 \times 10^{-7}$
		RE 244-BS†	$1.2 \times 10^{-8}$	$2.7 \times 10^{-7}$
		RE 642-BS†	$1.3 \times 10^{-8}$	$9.5 \times 10^{-7}$
		RE 86-BS†	$1.1 \times 10^{-8}$	$2.7 \times 10^{-6}$
		Verapamil	$5.0 \times 10^{-5}$	$> 10^{-4}$
		D 600 (methoxyverapamil)	$1.2 \times 10^{-5}$	$> 10^{-4}$
		D-Diltiazem	$3.4 \times 10^{-5}$	$> 10^{-3}$
		L-Diltiazem	$1.0 \times 10^{-5}$	$> 10^{-3}$
Non-Steroidal Anti-Inflammatory Agents		Indomethacin	$1.0 \times 10^{-8}$	$4.0 \times 10^{-6}$
		Ibuprofen	$1.4 \times 10^{-7}$	$> 10^{-4}$
		Meclofenamate	$1.0 \times 10^{-4}$	
		Indobufen	$5.0 \times 10^{-5}$	$> 10^{-4}$
		Flunixin meglumine	$1.0 \times 10^{-8}$	$1.2 \times 10^{-5}$
		Acetaminophen	$7.0 \times 10^{-7}$	$> 10^{-4}$
		Acetylsalicylic acid	$1.3 \times 10^{-7}$	$> 10^{-4}$
		Diclofenac sodium	$5.0 \times 10^{-4}$	$> 10^{-3}$
		Phenylbutazone	$> 10^{-4}$	
		Naproxen	$> 10^{-4}$	
Antibiotics		Puromycin	$5.6 \times 10^{-9}$	$4.6 \times 10^{-6}$
		Fovocamycin	$7.8 \times 10^{-8}$	$2.0 \times 10^{-6}$
		Actinomycin	$> 10^{-4}$	
		Rifampicin	$> 10^{-4}$	
Methylxanthines		Caffeine	$> 10^{-4}$	
		Theophylline	$> 10^{-4}$	
		3-Isobutyl-1-methylxanthine	$5.0 \times 10^{-5}$	$> 10^{-4}$
		1,3-Diallylxanthine	$1.0 \times 10^{-5}$	$> 10^{-3}$
		1,3-Di-N-propylxanthine	$1.6 \times 10^{-5}$	$5.8 \times 10^{-4}$
Metabolic Inhibitors and Miscellaneous		Cycloheximide	$> 10^{-4}$	
		Colchicine	$> 10^{-4}$	
		6-Benzylaminopurine riboside	$7.0 \times 10^{-7}$	$4.0 \times 10^{-5}$
		5-Fluorouracil	$> 10^{-4}$	
		6-Mercaptopurine	$> 10^{-4}$	

\* For further details of Methodology see Bender *et al.* (1980, 1981).

† RE 244-BS 4-(2'-hydroxyethyl-2"-hydroxypropyl-amino)-2,7-bis-(2-methyl-morpholino)-6-phenyl-pteridin. RE 642-BS 2,2'-(4,8-bis-diethylamino)-pyrimido, 5,4-D/-pyrimidin-2,6-diyl)-di-(2-methoxyethyl)-imino/diethanol. RE 86-BS 1,2'-(2,7-dimorpholino-6-phenyl-4-pteridinyliimino)-2-propanol-ethanol.

‡ Less than 10% inhibition of uptake at  $10^{-4}$  M.

agonists. Several such effects on adenosine uptake.

lators were tested as Dilazep, hexobendine, free analogs of dipyridamole and RE 86-BS. of adenosine uptake  $< 10^{-8}$  M. Papaverine is less potent. "m antagonists" coron methoxyverapamil are weak inhibitors of uptake  $> 10^{-5}$  M. This antagonists implies that dipyridamole and dilazep have an effect on membrane permeability.

es\*

IC<sub>50</sub> (M)

$1.1 \times 10^{-5}$   
 $1.0 \times 10^{-5}$   
 $1.0 \times 10^{-4}$   
 $> 10^{-4}$   
 $1.5 \times 10^{-4}$   
 $> 10^{-4}$   
 $1.1 \times 10^{-5}$   
 $6.0 \times 10^{-5}$   
 $> 10^{-4}$   
 $7.0 \times 10^{-7}$   
 $> 10^{-4}$   
 $5.6 \times 10^{-6}$   
 $5.0 \times 10^{-5}$   
 $8.5 \times 10^{-7}$   
 $3.5 \times 10^{-5}$   
 $5.0 \times 10^{-4}$   
 $4.0 \times 10^{-5}$   
 $6.0 \times 10^{-5}$   
 $7.0 \times 10^{-5}$   
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 $1.4 \times 10^{-6}$   
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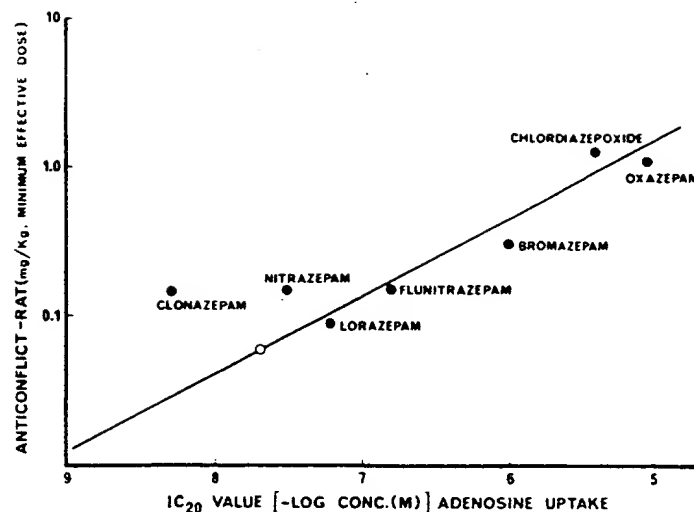


Fig. 2. Correlation between *in vitro* inhibition of adenosine uptake by rat brain cortical synaptosomes and *in vivo* anti-conflict effects (data from Cook & Sepinwall, 1975) for benzodiazepines. Pearson correlation coefficient  $r = 0.698$ .

Some of the coronary vasodilators which inhibit adenosine uptake (papaverine, prenylamine) have central depressant effects. Other potent adenosine uptake inhibitors, such as dipyridamole, which do not possess anxiolytic or sedative activity when administered systemically (Davies *et al.*, 1980) may be unable to cross the blood-brain barrier. Dipyridamole also possesses an ability to inhibit the release of adenosine (Fredholm *et al.*, 1980). The headaches that can occur during the initiation of dipyridamole therapy may be a result of elevated adenosine levels in the blood supply to cerebral arterial vessels. Adenosine is a potent dilator of such vessels (Phillis & Wu, 1981) and dilatation of cerebral vessels with an increased amplitude of pulsation in the arteries is known to be implicated in the aetiology of migraine headaches.

It should be noted that several of the agents which are classified under different headings in Table 1 can increase coronary blood flow and enhance the actions of adenosine on the coronary blood vessels. These

include the tricyclic antidepressants (Jefferson, 1975), indomethacin and ibuprofen (Lefer & Crossley, 1979; Glenn & Horan, 1981), the phenothiazines (Shamsi *et al.*, 1971) and benzodiazepines (Ikram *et al.*, 1973).

#### Non-steroidal anti-inflammatory drugs

Our interest in these agents was sparked by a report that indomethacin is a potent inhibitor of rat brain phosphodiesterase (Weinryb *et al.*, 1972).

Several of the drugs in this category were found to be potent inhibitors of adenosine uptake by rat brain synaptosomes including indomethacin, flunixin meglumine (Banamine) and ibuprofen. Others, meclofenamic acid, indobufen, diclofenac sodium (Voltaren), phenylbutazone and naproxen were only weakly inhibitory. Acetylsalicylic acid and acetaminophen have been difficult to categorize with respect to potency. Although both substances had submicromolar  $IC_{50}$  values, their dose-response curves were extremely shallow, making it impossible to reach an  $IC_{50}$  value within the range of concentrations ( $10^{-9}$ – $10^{-4}$  M) tested.

The observation that some non-steroidal anti-inflammatory drugs can inhibit adenosine uptake is both novel and exciting. It is possible that actions of these agents which were attributed to an inhibition of prostaglandin synthesis may have been due to potentiation of adenosine levels. Previous reports of calcium antagonism by substances in this group (Northover, 1977) may have been a result of an adenosine-mediated inhibition of calcium influx into the tissues.

#### Antibiotics

Two of the four compounds tested in this group (puromycin and toyocamycin) are structurally closely related to adenosine. Both were potent inhibitors of adenosine uptake with  $IC_{50}$  values of  $5.6 \times 10^{-9}$  M and  $7.8 \times 10^{-8}$  M for puromycin and toyocamycin, respectively. Actinomycin and rifampicin were virtually inactive.

Puromycin has been extensively used in experiments on memory formation and retention. Following

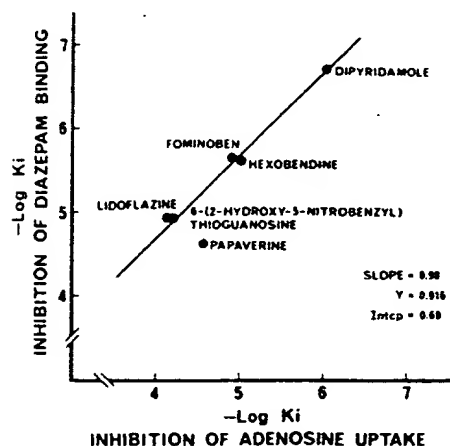


Fig. 3. Correlation between  $K_i$  values for the inhibition of  $^3H$ -diazepam binding and for the inhibition of adenosine uptake by various adenosine uptake inhibitors. Slope 0.98; Correlation coefficient  $r = 0.916$  ( $0.01 > P > 0.00$ ). From Wu *et al.* (1981).

intracranial administration of this compound, animals and birds appear to be sedated and ataxic (R. A. Barraco, personal communication).

### Methylxanthines

3-Isobutyl-1-methylxanthine, 1,3-diallylxanthine and 1,3-di-N-propylxanthine were relatively effective inhibitors of adenosine uptake with  $IC_{20}$  values in the  $10^{-5}$ – $10^{-4}$  M range. Caffeine and theophylline were less effective.

These results can be compared with those obtained in pharmacological studies. Whereas caffeine and theophylline act as reliable adenosine antagonists, the findings with isobutylmethylxanthine are less consistent. In some instances it potentiates the action of adenosine but at other times it antagonizes adenosine (Phillis *et al.*, 1979). Likewise in studies on mouse locomotor activity, where caffeine and theophylline had an excitant action, isobutylmethylxanthine elicited only behavioral depression (Snyder *et al.*, 1981). We propose that the adenosine uptake inhibiting activity of isobutylmethylxanthine may be sufficient to overcome its actions as an adenosine antagonist, thus giving this compound a behaviorally depressant action. With caffeine and theophylline, adenosine antagonism will be the predominant effect as these compounds are less active uptake inhibitors and they will therefore act as stimulants. At low doses, caffeine can have a central depressant action and this could be a reflection of differing slopes for its activities as an antagonist or potentiator of adenosine action.

### Morphine

An association between morphine and adenosine was initially suggested by the observation that methylxanthines antagonize the depressant effects of morphine on acetylcholine release from the cerebral cortex (Jhamandas & Sawynok, 1976). Subsequent studies have shown that theophylline antagonizes the depressant effects of morphine on striatal neurons (Stone & Perkins, 1979) and that morphine can enhance the rate of release of labelled adenine nucleotides from the cerebral cortex (Phillis *et al.*, 1980). Theophylline increases the  $LD_{50}$  of morphine in mice and decreases the inhibitory effect of morphine on synaptosomal uptake of calcium (Brailowsky *et al.*, 1981). The purine-release enhancing actions of morphine could be due either to an increase in purine release or to an inhibition of adenosine uptake. *In vitro* experiments have demonstrated that morphine enhances the potassium-evoked release of  $^3H$ -purines from brain prisms pre-incubated in  $^3H$ -adenosine (P. H. Wu, J. W. Phillis and H. Yuen, unpublished observations). Comparisons of the release elicited by morphine and dipyridamole suggested that even though morphine is a weak inhibitor of adenosine uptake ( $IC_{20} \approx 10^{-4}$  M), this effect is unlikely to have made a significant contribution to the release of adenosine by morphine. Rather, morphine is apparently able to enhance the release of adenosine by a direct influence on the releasing mechanism.

### CONCLUSIONS

Experimental observations on *in vivo* neurons have shown that adenosine's primary action is to reduce

the release of other transmitters through an action on presynaptic nerve terminals. Drugs which block the uptake and metabolism of adenosine depress the firing of central neurons and potentiate adenosine's depressant action. Adenosine receptor antagonists excite central neurons. These observations suggest that the excitability of central neurons is subject to regulation by endogenously released adenosine.

A survey of several groups of centrally active drugs has demonstrated that many sedative, anxiolytic, anti-convulsant, antidepressant and analgesic compounds are potent inhibitors of adenosine uptake by rat brain synaptosomes. These include phenothiazines, benzodiazepines, tricyclic antidepressants, steroids, some of the non-steroidal anti-inflammatory drugs and the antibiotics puromycin and toyocamycin. Potentiation of the effects of endogenously released adenosine may be an important factor in the central actions of these compounds. Morphine enhances adenosine release from central preparations and adenosine mediates the depressant effects of morphine on acetylcholine release from central nerve terminals. Inhibition of adenosine uptake makes a small contribution to the elevated adenosine levels and a direct releasing action of morphine appears to be the more important factor.

Our observations emphasize the critical role that adenosine appears to play in central nervous system functioning and suggest that the development of more potent potentiators and antagonists of adenosine may generate valuable new approaches to the treatment of psychiatric and neurological disorders.

### REFERENCES

- BALDESSARINI R. J. (1980) Drugs and the treatment of psychiatric disorders. In *The Pharmacological Basis of Therapeutics* (Edited by GILMAN A. G., GOODMAN L. S. & GILMAN A.), pp. 391–447. Macmillan, New York.
- BEER B., CHASIN M., CLODY D. E., VOGEL J. R. & HOROVITZ Z. P. (1972) Cyclic adenosine monophosphate phosphodiesterase in brain: effect in anxiety. *Science, N.Y.* 176, 428–430.
- BENDER A. S., WU P. H. & PHILLIS J. W. (1980) The characterization of  $^3H$ -adenosine uptake into rat cerebral cortical synaptosomes. *J. Neurochem.* 35, 629–640.
- BENDER A. S., WU P. H. & PHILLIS J. W. (1981) The rapid uptake and release of [ $^3H$ ]adenosine by rat cerebral cortical synaptosomes. *J. Neurochem.* 36, 651–660.
- BIEGON A. & SAMUEL D. (1979) Binding of labeled antidepressants to rat brain tissue. *Biochem. Pharmacol.* 28, 3361–3366.
- BRAILOWSKY S., GUERRERO-MUÑOZ F., LUJÁN M. & SHKUROVICH M. (1981) Morphine-theophylline interaction: antagonism or facilitation? *Br. J. Pharmacol.* 73, 887–892.
- BRANISTEANU D. D., HAULICA I. D., PROCA B. & NHUE B. G. (1979) Adenosine effects upon transmitter release parameters in the  $Mg^{2+}$ -paralyzed neuromuscular junction of frog. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmacol.* 308, 273–279.
- BUDAY P. V., CARR C. J. & MIYA T. S. (1961) A pharmacologic study of some nucleosides and nucleotides. *J. Pharm. Pharmacol.* 13, 290–299.
- BUDD K. (1978) Psychotropic drugs in the treatment of chronic pain. *Anaesthesia* 33, 531–534.
- CARDINALI D. P. (1980) Methylxanthines: possible mechanisms of action in brain. *Trends Pharmacol. Sci.* 1, 405–407.
- CHARPENTIER J. (1966) Nouvelle recherche sur le mécanisme d'action de certains analgésiques centraux (acide

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id retention. Following

- acetylsalicylique, imipramine, iminostilbene. *Psychopharmacology*, **10**, 126-147.
- CLANACHAN A. S., HAMMOND J. R. & PATERSON A. R. P. (1981) Coronary vasodilator and benzodiazepine inhibition of site-specific binding of nitrobenzylthioinosine, an inhibitor of nucleoside transport, to human erythrocytes. *Br. J. Pharmac.* **74**, 835-836P.
- COOK L. & SEPINWALL J. (1975) Behavioral analysis of the effects and mechanisms of action of benzodiazepines. *Adv. Biochem. Psychopharmac.* **14**, 1-28.
- CURRY S. H., MARSHALL J. H. L., DAVIS J. M. & HANOWSKY D. S. (1970) Chlorpromazine plasma levels and effects. *Archs gen. Psychiat.* **22**, 289-296.
- DALY J. (1977) *Cyclic Nucleotides in the Nervous System*. Plenum Press, New York.
- DAVIES L. P., COOK A. F., POONIAN M. & TAYLOR K. M. (1980) Displacement of [ $^3$ H]diazepam binding in rat brain by dipyridamole and by 1-methylisoguanosine, a marine natural product with muscle relaxant activity. *Life Sci.* **26**, 1089-1095.
- FELDBERG W. & SHERWOOD S. L. (1954) Injections of drugs into the lateral ventricle of the cat. *J. Physiol., Lond.* **123**, 148-167.
- FREDHOLM B. B. (1980) Are methylxanthine effects due to antagonism of endogenous adenosine? *Trends Pharmac. Sci.* **1**, 129-132.
- FREDHOLM B. B., SOLLEVI A., VERNET L. & HEDQVIST P. (1980) Inhibition by dipyridamole of stimulated purine release. *Naunyn Schmiedeberg's Arch. exp. Path. Pharmac.* **313**, R18.
- GINSBORG B. L. & HIRST G. D. S. (1972) The effect of adenosine on the release of transmitter from the phrenic nerve of the rat. *J. Physiol., Lond.* **224**, 629-645.
- GLENN T. M. & HORAN J. (1981) Modification of adenosine induced changes in coronary flow by methylprednisolone and indomethacin. *Fedn Proc. Fedn Am. Soc. exp. Biol.* **40**, 706.
- GLENN E. M., MILLER W. L. & SCHLAGEL C. A. (1963) Metabolic effects of adrenal-cortical steroids *in vivo* and *in vitro*: relationship to anti-inflammatory effects. *Recent Prog. Horm. Res.* **19**, 107-191.
- GOODSELL E. B., STEIN H. H. & WENZKE K. J. (1971) 8-Substituted theophyllines. *In vitro* inhibition of 3',5'-cyclic adenosine monophosphate phosphodiesterase and pharmacological spectrum in mice. *J. med. Chem.* **14**, 1202-1205.
- GREDDEN J. F. (1974) Anxiety or caffeinism: a diagnostic dilemma. *Am. J. Psychiat.* **131**, 1089-1092.
- GREDDEN J. F., FONTAINE P., LUBETSKY M. & CHAMBERLIN K. (1978) Anxiety and depression associated with caffeinism among psychiatric inpatients. *Am. J. Psychiat.* **135**, 963-966.
- GREEN A. L. (1967) Activity correlations and the mode of action of aminoalkylphenothiazine tranquilizers. *J. Pharm. Pharmac.* **19**, 207-208.
- HARMS H. H., WARDEH G. & MULDER A. H. (1978) Adenosine modulates depolarization-induced release of [ $^3$ H]noradrenaline from slices of rat brain neocortex. *Eur. J. Pharmac.* **49**, 305-308.
- HARMS H. H., WARDEH G. & MULDER A. H. (1979) Effects of adenosine on depolarization-induced release of various radiolabelled neurotransmitters from slices of rat corpus striatum. *Neuropharmacology* **18**, 577-580.
- HESS S. M., CHASIN M., FREE C. A. & HARRIS D. N. (1975) Modulators of cyclic AMP systems. *Adv. Biochem. Psychopharmac.* **14**, 153-167.
- HOLLINS C. & STONE T. W. (1980) Adenosine inhibition of  $\gamma$ -aminobutyric acid release from slices of rat cerebral cortex. *Br. J. Pharmac.* **69**, 107-112.
- HOPKINS S. V. (1973) The potentiation of the action of adenosine on the guinea-pig heart. *Biochem. Pharmac.* **22**, 341-348.
- HUANG M. & DALY J. W. (1972) Accumulation of cyclic adenosine monophosphate in incubated slices of brain tissue. I. Structure-activity relationships of agonists and antagonists of biogenic amines and of tricyclic tranquilizers and antidepressants. *J. med. Chem.* **15**, 458-462.
- IKRAM H., RUBIN A. P. & JEWKES R. F. (1973) Effect of diazepam on myocardial blood flow of patients with and without coronary artery disease. *Br. Heart J.* **35**, 626-630.
- JASANI M. K. (1979) Anti-inflammatory steroids: mode of action in rheumatoid arthritis and homograft reaction. *Handbk expl Pharmac.* **50**, 599-660.
- JEFFERSON J. W. (1975) A review of the cardiovascular effects and toxicity of tricyclic antidepressants. *Psychosom. Med.* **37**, 160-179.
- JHAMANDAS K. & SAWYONOK J. (1976) Methylxanthine antagonism of opiate and purine effects on the release of acetylcholine. In *Opiates and Endogenous Opioid Peptides* (Edited by KOSTERLITZ H. W.), pp. 161-168. Elsevier-North Holland, Amsterdam.
- KINARD C. O., CHANG S. S. & DAVIS J. M. (1978) Plasma levels of antidepressants and clinical response. In *Clinical Neuropharmacology* (Edited by KLAUWANS H. L.), pp. 103-122. Raven Press, New York.
- KODAMA T., MATSUKADO Y., SUZUKI T., TANAKA S. & SHIMIZU H. (1971) Stimulated formation of cyclic AMP by desipramine in brain slices. *Biochim. biophys. Acta* **252**, 165-170.
- KURODA Y. (1978) Physiological roles of adenosine derivatives which are released during neurotransmission in mammalian brain. *J. Physiol., Paris* **74**, 463-470.
- LEFER A. M. & CROSSLEY K. (1979) Optimal dose of ibuprofen in acute myocardial ischemia in the cat. *Circulation Shock* **6**, 182-183.
- LEKIĆ D. (1977) Presynaptic depression of synaptic response of Renshaw cells by adenosine 5'-monophosphate. *Can. J. Physiol. Pharmac.* **55**, 1391-1393.
- LIPPA A. S., COUPET J., GREENBLATT E. N., KLEPNER C. A. & BEER B. (1979) A synthetic non-benzodiazepine ligand for benzodiazepine receptors: a probe for investigating neuronal substrates of anxiety. *Pharmac. Biochem. Behav.* **11**, 99-106.
- MOLLER NIELSEN I., PEDERSEN V., NYMARK M., FRANCK K. F., BOECK V., FJALLAND B. & CHRISTENSEN A. V. (1973) The comparative pharmacology of flupenthixol and some reference neuroleptics. *Acta pharmac. tox.* **33**, 353-362.
- NORTHOVER B. J. (1977) Indomethacin—a calcium antagonist. *Gen. Pharmac.* **8**, 293-296.
- PALMER G. C. (1979) Interactions of anti-epileptic drugs on adenylate cyclase and phosphodiesterases in rat and mouse cerebrum. *Exp. Neurol.* **63**, 322-335.
- PHILLIS J. W. (1979) Diazepam potentiation of purinergic depression on central neurons. *Can. J. Physiol. Pharmac.* **57**, 432-435.
- PHILLIS J. W., EDSTROM J. P., ELLIS S. W. & KIRKPATRICK J. R. (1979) Theophylline antagonizes flurazepam-induced depression of cerebral cortical neurons. *Can. J. Physiol. Pharmac.* **57**, 917-920.
- PHILLIS J. W., EDSTROM J. P., KOSTOPOULOS G. K. & KIRKPATRICK J. R. (1979) Effects of adenosine and adenine nucleotides on synaptic transmission in the cerebral cortex. *Can. J. Physiol. Pharmac.* **57**, 1289-1312.
- PHILLIS J. W., JIANG Z. G., CHELACK B. J. & WU P. H. (1980) The effect of morphine on purine and acetylcholine release from rat cerebral cortex: evidence for a purinergic component in morphine's action. *Pharmac. Biochem. Behav.* **13**, 421-427.
- PHILLIS J. W. & KOSTOPOULOS G. K. (1975) Adenosine as a putative transmitter in the cerebral cortex: Studies with potentiators and antagonists. *Life Sci.* **17**, 1085-1094.
- PHILLIS J. W., SIEMENS R. K. & WU P. H. (1980) Effects of

- ated slices of brain  
ships of agonists and  
nd of tricyclic trans-  
med. Chem. 15, 458.
- R. F. (1973) Effect of  
v of patients with and  
Br. Heart J. 33.
- ory steroids: mode of  
homograft reaction
- of the cardiovascular  
depressants. Psych-
- Methylxanthine an-  
ects on the release of  
enous Opioid Peptides  
p. 161-168. Elsevier
- J. M. (1978) Plasma  
al response. In Clin-  
Klawans H. L., pp.
- T., TANAKA S. & Su-  
on of cyclic AMP by  
m. biophys. Acta 252.
- s of adenosine deriva-  
neurotransmission in  
s 74, 463-470.
- Optimal dose of ibu-  
mia in the cat. Circu-
- ssion of synaptic re-  
nosine 5'-monophos-  
s, 1391-1393.
- E. N., KLEPNER C. A.  
benzodiazepine ligand  
robe for investigating  
rmac. Biochem. Behav.
- YMARK M., FRANCE  
CHRISTENSEN A. V.  
ology of flupenthixol  
icta pharmac. tox. 31.
- in—a calcium antag-
- anti-epileptic drugs on  
esterases in rat and  
322-335.
- atiation of purinergic  
J. Physiol. Pharmac.
- W. & KIRKPATRICK  
gonizes flurazepam  
tical neurons. Can. J.
- oulos G. K. & Kirk-  
enosine and adenine  
n in the cerebral cor-  
289-1312.
- K B. J. & Wu P. H.  
purine and acetylcho-  
x: evidence for a pur-  
action. Pharmac. Bio-
- K. (1975) Adenosine  
rebral cortex: Studies  
Life Sci. 17, 1085.
- P. H. (1980) Effects of  
diazepam on adenosine and acetylcholine release from  
rat cerebral cortex: further evidence for a purinergic  
mechanism in action of diazepam. Br. J. Pharmac. 70,  
341-348.
- PHILLIS J. W. & Wu P. H. (1981) The role of adenosine and  
its nucleotides in central synaptic transmission. Prog.  
Neurobiol. 16, 187-239.
- POLE P., BONETTI E. P., PIERI L., CUMIN R., ANGIOI R. M.,  
MÖHLER H. & HAEFELY W. E. (1981) Caffeine antagon-  
izes several central effects of diazepam. Life Sci. 28,  
2265-2275.
- RIBEIRO J. A., SÁ-ALMEIDA A. M. & NAMORADO J. M. (1979)  
Adenosine and adenosine triphosphate decrease <sup>45</sup>Ca  
uptake by synaptosomes stimulated by potassium. Bio-  
chem. Pharmac. 28, 1297-1300.
- SATTIN A., STONE T. W. & TAYLOR D. A. (1978) Biochemi-  
cal and electropharmaceutical studies with tricyclic anti-  
depressants in rat and guinea-pig cerebral cortex. Life  
Sci. 23, 2621-2626.
- SELYE H. (1942) Studies concerning the correlation between  
anesthetic potency, hormonal activity and chemical  
structure among steroid compounds. Anesth. Analg. 21,  
41-47.
- SHAMSI M. A., KULSHRESTHA V. K. & DHAWAN K. N.  
(1971) Correlation of chemical structure of phenothia-  
zines with their coronary dilator and antiarrhythmic ac-  
tivities. Jap. J. Pharmac. 21, 747-754.
- SNYDER S. H., KATIMS J. J., ANNAU Z., BRUNS R. F. &  
DALY J. W. (1981) Adenosine receptors and behavioral  
actions of methylxanthines. Proc. natn. Acad. Sci. U.S.A.  
78, 3260-3264.
- STANTON H. C., TAYLOR D. P. & RIBLET L. A. (1981) Bus-  
pirone—an anxiolytic drug with dopaminergic  
action. In The Neurobiology of the Nucleus Accumbens  
(Edited by CHRONISTER R. B. & DeFRANCE J. F.), pp.  
316-321. Haer Institute, Brunswick.
- STONE T. W. & PERKINS M. N. (1979) Is adenosine the  
mediator of opiate action on neuronal firing rate?  
Nature, Lond. 281, 227-228.
- WEINRYB I., CHASIN M., FREE C. A., HARRIS D. N., GOL-  
DENBERG H., MICHEL I., PAIK V. S., PHILLIPS M., SAM-  
NIEGO S. & HESS S. M. Effects of therapeutic agents on  
cyclic AMP metabolism in vitro. J. Pharm. Sci. 61,  
1556-1566.
- WILLIAMS M., RISLEY E. A. & HUFF J. R. (1981) Interaction  
of putative anxiolytic agents with central adenosine  
receptors. Can. J. Physiol. Pharmac. 59, 897-900.
- WU P. H., PHILLIS J. W. & BENDER A. S. (1981) Do benzo-  
diazepines bind at adenosine uptake sites in CNS? Life  
Sci. 28, 1023-1031.